Potential Role of Endothelin-1 in Pulmonary Fibrosis

From the Bench to the Clinic

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Endothelin-1 (ET-1) plays a central role in lung fibrosis. It is released in the lung at low concentrations from the endothelium, epithelium, and vascular smooth muscle cells and orchestrates a variety of effects. In the context of wound healing, ET-1 acts with other profibrotic mediators to recruit fibroblasts and allow for their differentiation to contractile myofibroblasts. These specialized cells in turn lay down fibrotic tissue and contract at the site of lesions to restore tissue integrity. Apoptosis and reversion to quiescence ensues. However, in diseases of the lung such as idiopathic pulmonary fibrosis (IPF), the fibrotic response is uncontrolled. Progressive injury to lung tissue, isolated both temporally and geographically, is uncontrolled and eventually causes enough tissue damage to alter pulmonary architecture and compromise function. The initiating mechanisms are as of yet largely unknown; however, ET-1 has clearly emerged as a key mediator of this disease. Here, a comprehensive overview of the role of ET-1 in fibrosis is given. A guided perspective begins from the scope of its various molecular interactions to its many cellular processes, and finally to the implications of these functions in IPF.

Keywords: lungs, human; endothelin receptor antagonist; idiopathic pulmonary fibrosis; extracellular matrix; myofibroblast

In the face of excessive fibrosis, specialized organs such as the kidney, liver, heart, and lungs stand to lose significant portions of functional tissue (1). Idiopathic pulmonary fibrosis (IPF) is a progressive disease of the lung that is thought to result from an abnormal response to wound healing (2). To achieve a successful outcome in treating patients with IPF, it is essential to achieve a comprehensive understanding of the cellular and molecular interactions at play. Indeed, in determining the key components separating physiologic and pathologic wound repair, potential targets for therapy have come to light. Endothelin-1 (ET-1) has emerged as a potential pharmacologic target in recent years and appears to hold promise in future IPF treatment. Elevated expression and release of ET-1 in the lungs has been well documented in both humans (3–5) and in animal models (6–9), and its profibrotic pathways continue to be unraveled.

ET-1 AND ITS RECEPTORS

Of the endothelin family, three primary isoforms have been isolated: ET-1, ET-2, and ET-3, of which ET-1, a 21–amino acid peptide, is the most abundant (10). In addition to playing a key role in many aspects of fibrosis, ET-1 is also a mitogen and one of the most powerful endogenous vasoconstrictors known (11).

ET-1 is synthesized as an inactive 212–amino acid preprohormone, preproET-1 (10). The latter is cleaved by endopeptidases to the 39–amino acid, big ET-1 (10). A final cleavage step, performed by endothelin converting enzyme-1 (ECE-1), converts big ET-1 to the 21–amino acid product ET-1 (10). This last step is associated with a 500-fold increase in biological activity in *in vitro* assays (2). Recent data has shown that big ET-1 can be cleaved to a biologically active intermediate ET-1 (1–31) by the action of chymase (16). The levels of ET-1 compounds are elevated in IPF, with the expression patterns of big ET-1 and ECE-1 significantly correlated to disease activity (13).

ET-1 is released in the normal lung by endothelial cells, epithelial cells, and mesenchymal cells (17). In fibrotic and diseased states, ET-1 is additionally secreted by inflammatory cells such as macrophages and neutrophils (13), in addition to fibroblasts and myofibroblasts (14). As ET-1 is not stored in the cell, the regulation of its release occurs at the level of transcription (10). Once it is produced, the guided intracellular transport and secretion of ET-1 via vesicles is important in allowing this compound to carry out directional autocrine and paracrine signaling (10).

ET-1 binds to two G protein–coupled receptors, ET receptor A (ET_A) and ET receptor B (ET_B). ET_A receptors are found primarily on mesenchymal cell types such as vascular and airway smooth muscle cells (10) and fibroblasts (2). ET_B receptors, although primarily found on the endothelium (10), are also expressed on mesenchymal cells (2). These two receptor subtypes work together, although they can also carry out some functions independently of one another. For example, the ET_A receptor plays the predominant role in cell proliferation (2), vasoconstriction (2), proinflammatory (18) and autocrine (10) actions, whereas the ET_B receptor predominantly plays an ET-1 negative feedback clearance role (2), along with specialized actions such as airway bronchoconstriction (10) and vascular release of nitric oxide (2). The profibrotic effects of ET-1 typically involve both receptor subtypes (1).

MOLECULAR INTERACTIONS AND BIOLOGICAL PATHWAYS

In the context of fibrosis, there are a handful of especially important biological mediators whose pathways interact closely with those of ET-1. Such compounds include transforming growth factor-β (TGF-β), connective tissue growth factor (2), TNF-α, and a variety of cytokines such as IL-1 (13). The process of fibrosis is remarkably similar in different organs (1). This universality implies that many of these biological interactions are common in different organs throughout the body (1).

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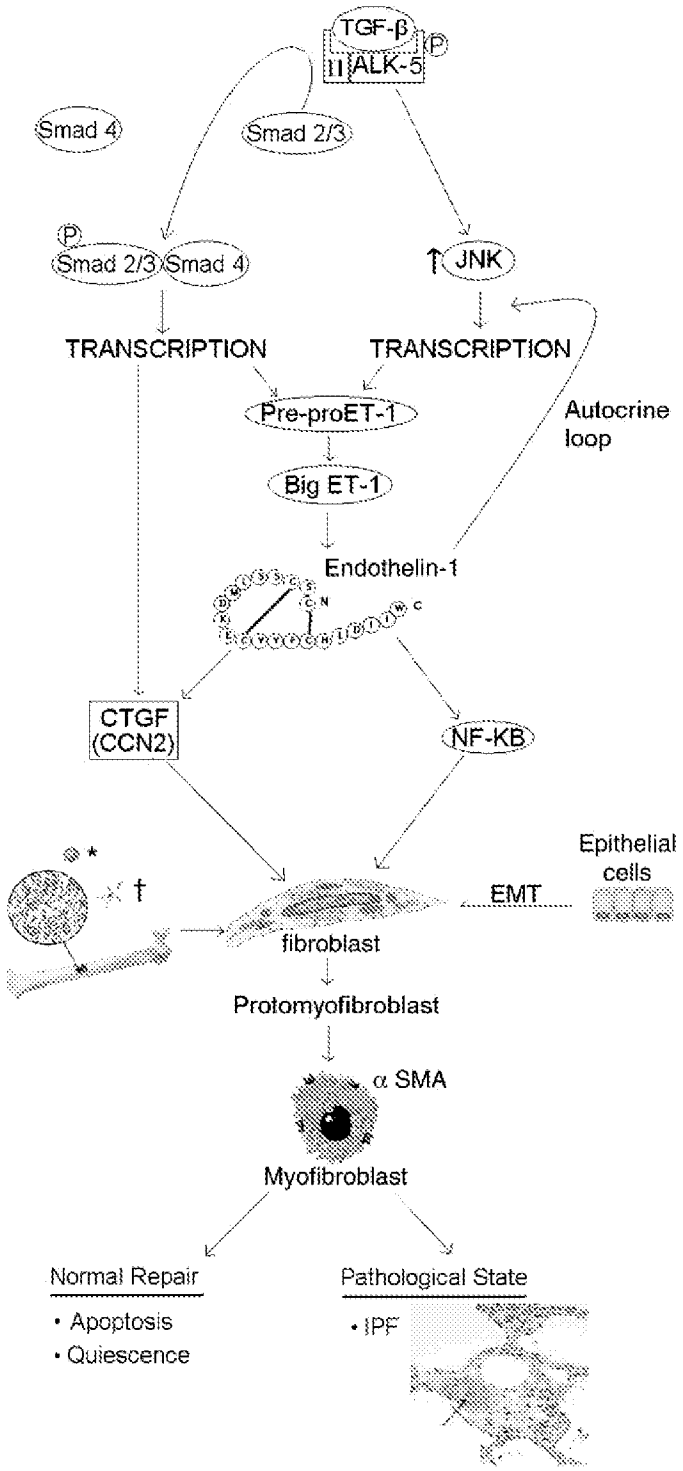

Figure 1. Schematic representation of endothelin-1 (ET-1) activation, interaction, and target in pulmonary fibrosis. The transforming growth factor-β (TGF-β) cascade elicits transcription of ET-1, via both Smad-dependent and Smad-independent pathways. ET-1 in turn increases NF-κB and, along with TGF-β, also increases connective tissue growth factor 2 (CTGF/CCN2). ET-1 also increases its own production via an autocrine loop. These potent profibrotic mediators allow fibroblasts to differentiate into myofibroblasts. The latter adhere and contract at lesion sites, and produce fibrotic extracellular matrix components. The normal healing process requires that these specialized cells subsequently undergo apoptosis. If fibrotic activity is uncontrolled, pathologic fibrosis characteristic of idiopathic pulmonary fibrosis (IPF) occurs leading to pulmonary remodeling and dysfunction. α-SMA, α–smooth muscle actin; EMT, epithelial to mesenchymal transition; JNK, c-Jun N-Terminal kinase. *Hematopoietic stem cell. †Stromal stem cell.

Along with ET-1, TGF-β and connective tissue growth factor (known as CCN2) are the predominant profibrotic mediators (2, 14). These three mediators interact as members of common pathways, with CCN2 downstream to ET-1 and TGF-β, and the former acting both downstream and in concert with TGF-β (2). Indeed, the degree of up-regulation of ET-1 and TGF-β mirror one another in the same disease processes (1).

TGF-β induces the expression of ET-1, both in normal and in fibrotic lung fibroblasts, in a Smad-dependent, c-Jun N-terminal kinase (JNK)-dependent fashion (2). In fibrotic fibroblasts, however, there is evidence of additional autocrine activity at play, whereby released ET-1 induces JNK activation to further increase expression of ET-1 (2). The many profibrotic effects of TGF-β on fibroblasts, including fibroblast activation, differentiation, and the expression of profibrotic genes such as type-1 collagen and fibronectin, appear to involve the activity of ET-1 (19). In summary, ET-1 appears to be up-regulated largely by upstream TGF-β in the fibrotic pathway. Once expressed, ET-1 increases its own expression via an autocrine loop, in addition to acting synergistically with TGF-β to mediate fibrosis (Figure 1).

THE WOUND REPAIR PROCESS AND FIBROSIS

Fibroblasts are spindle-shaped mesenchymal-derived cells (20). They are abundant throughout the body, particularly in specialized organs containing high levels of connective tissue such as the kidneys, liver and lungs (1, 2). Fibroblasts play the essential role of regulating the extracellular matrix (ECM). This includes the maintenance of ECM fluid volume and pressure properties, as well as carefully regulating its composition (20). Fibroblasts are pivotal in the wound healing process in response to tissue injury (20). Thus, fibroblasts, in their many phenotypic forms, also play a central role in IPF as any abnormalities in fibroblastic function carry very serious clinical consequences. The importance of the fibroblast is put into perspective by the suggestion that up to half of all deaths are associated with fibrosing conditions (20). Enhancement of mast cell–derived metalloproteinase and chymase expression has been demonstrated in interstitium remodeling characteristic of pulmonary fibrosis (21).

The fibroblast is an incredibly active cell type, constantly synthesizing and degrading components of the extracellular matrix (14). Fibroblasts synthesize a variety of compounds, including collagens, fibronectin, laminin, and proteoglycans, at rapid rates (20). In fact, one metabolically active fibroblast is capable of producing up to an estimated 3.5 million procollagen molecules per day (20). Fibroblasts also release regulatory compounds such as the matrix-degrading matrix metalloproteinases (MMPs) as well as inhibitors of MMPs (20). These regulatory secretions allow fibroblasts to maintain an appropriate extracellular matrix makeup in a variety of settings, both physiologic and pathologic. In response to tissue damage, local fibroblast numbers increase substantially; ET-1 is involved in this chemotactic process (22). Fibroblastic precursors can be local, such as resident mesenchymal stem cells, or can come from bone marrow–derived fibrocytes circulating in the bloodstream (14, 20).

A third source of growing interest is a process known as epithelial–mesenchymal transition (EMT), whereby resident

epithelial cells actually take on fibroblast-like features (23). This remarkable plasticity is accompanied by a contractile phenotype and the loss of epithelial cell properties (23). Although most of the work on EMT thus far has employed *in vitro* models on removed epithelial cell lines (24), there is recent and growing evidence that ET-1 is involved in EMT via the stimulation of TGF-β (25). Briefly, ET-1 can activate TGF-β in a G protein–dependent manner in alveolar epithelial cell lines to affect the loss of an epithelial marker and gain the mesenchymal α–smooth muscle actin fiber (25).

Many of the fibroblasts at the site of lesions differentiate to a contractile phenotype, upon which they are referred to as myofibroblasts. This differentiation process has also been demonstrated to be ET-1 dependent (26). The binding of ET-1 to its receptor on mesenchymal cells promotes two responses, one rapid and one much slower. The rapid response is a G protein–mediated increase in intracellular calcium, causing smooth muscle contraction; the slower response, particularly important to pulmonary fibrosis, elicits the up-regulation of matrix-modifying genes and ultimately leads to cell differentiation (Figure 1) (2).

Fibroblasts, intermediate protomyofibroblasts, and myofibroblasts are the primary active cells at sites of wound repair (27). Myofibroblasts display specialized expression of α–smooth muscle actin stress fibers and specialized integrins which confer contractile properties (20). This allows myofibroblasts to adhere to the matrix and contract to close lesions in the ECM. The production and release of specific matrix components also becomes altered, with a shift toward the synthesis of collagen-1 and collagen-3 and an inhibition of MMP release (2). All of these effects have been demonstrated to be ET-1 dependent (2). Importantly, upon wound closure, these active profibrotic cells undergo cell apoptosis or revert back to noncontractile cell types (14).

Fibrosis, and even minor scarring, is physiologic and indeed critical to maintaining tissue structure; if acute, the fibrotic process acts to restore proper function of the organ. In pathologic states, however, the normal wound repair response is altered. Uncontrolled focal fibrosis occurs, and extensive scarring of specialized tissues results in permanently compromised organ function (15). The sequences that separate physiologic and pathologic wound repair remain to be resolved. It is interesting to note that in pulmonary fibrosis, fibroblasts appear to be resistant to apoptosis, whereas epithelial cells appear to have a higher susceptibility for apoptosis (14). This may help to explain the sequential fibrosis characteristic of IPF.

IDIOPATHIC PULMONARY FIBROSIS

IPF is a progressive and fatal disease characterized by extensive fibrosis and scarring of lung tissue from alveolar epithelial cell damage (2, 28). Over time, eventual architectural alterations in lung morphology lead to compromised gas exchange (14). IPF is one of the most common forms of interstitial lung disease encountered in clinical practice (28). As its name implies, the etiology of IPF is still largely unknown, which makes this disease incredibly difficult to treat. Indeed, the prognosis for patients with IPF is worse than most forms of cancer, with an average life expectancy of 3 years from diagnosis (15). IPF is best confirmed through lung biopsy, although secondary techniques such as CT scans and bronchoalveolar lavage fluid can also be helpful (15). The characteristic pattern of IPF is that of usual interstitial pneumonia (15).

The progression of organ damage in IPF was classically thought to be caused by chronic inflammation (15). It was thought that an initial stimulus or insult to the lung would chronically activate the inflammatory cascade, further damaging the lung tissue (20). An important landmark in IPF research was the eventual realization that chronic inflammation was not the source of disease (2). Rather, the inflammatory response acts more as a modifier of the primary fibrogenic response (2). Lung tissue from patients with IPF was discovered to display newer, active fibroblast foci interspersed among older fibrotic and scarred zones; this indicates a pattern of sequential, unrelated episodes of fibrosis (15). Early common misdiagnoses of IPF, coupled to pharmacologic studies concluding that anti-inflammatory therapy does not alleviate the symptoms of true IPF, has led to the current concept of IPF as a progressive fibrotic disease with an associated inflammatory response (15).

ET-1 IN IPF: DIRECT LINES OF EVIDENCE

The current notion of IPF is that it is primarily a process of sequential fibrosis rather than chronic inflammation. Consequently, ET-1 has been further implicated as an important mediator in the progression of this disease. As already mentioned, ET-1 is the principal effector of the many profibrotic roles of TGF-β such as the differentiation of fibroblasts to myofibroblasts (20), the production of altered ECM components (2), the inhibition of ECM degradation (2, 20) and the differentiation of epithelial cells into fibrotic mesenchymal cells (EMT) (Figure 1) (2, 25). Interestingly, the same signaling pathways involved in EMT are observed in IPF; ET-1 is implicated in both (25).

There is mounting experimental evidence from various human studies that implicate ET-1 in the progression of IPF. Analysis of serum from patients with IPF showed that these samples contained higher concentrations of ET-1 when compared with samples from control subjects (3). Subsequent immunohistochemical analysis of lung biopsies from patients with confirmed IPF were found to contain elevated big ET-1, ECE-1, and ET-1 expression when compared with normal lungs (13). The same study also demonstrated that big ET-1 and ECE-1 were found to co-localize in the lung in areas that correlated to disease activity (13). Similar ET-1 level patterns were also observed in bronchoalveolar lavage fluid analysis (4). Interestingly, the former study also demonstrated that inflammatory cells present in the lung during IPF, such as macrophages and neutrophils, both secrete ET-1, whereas this was not observed in the normal lung (13). In fact, alveolar macrophages spontaneously secrete ET-1 in IPF when compared with control subjects (5).

Further support for the role of ET-1 in lung fibrosis comes from animal studies. Bleomycin-induced pulmonary fibrosis in the rat results in increased big ET-1, ECE-1, ET-1, and even ET receptor expression (6–8). Also, transgenic mice that overexpress human pre–proET-1 and ET-1 transcriptional elements spontaneously develop progressive pulmonary fibrosis (9).

FUTURE DIRECTIONS

The ability to control the pathologic effects of ET-1 may influence the manner in which patients with IPF are treated in the coming years. Given the hierarchical dominance of TGF-β in fibrotic processes, it is not unreasonable to wonder why TGF-β is not more heavily targeted. It is simply because TGF-β is also a key inhibitor in immune responses and plays a role in tumor suppression (14). Rather than directly inhibiting TGF-β, ET-1 targeting inhibits the many TGF-β–dependent ET-1 effects in a safer manner, in addition to inhibiting the many events that ET-1 induces independently of TGF-β.

The production of ET-1 is a process of sequential enzymatic steps. Research has focused on the inhibition of the various key

enzymes involved in this pathway. ECE-1 is one such useful target. CGS 26303, for example, is one of many experimental nonselective ECE and endopeptidase inhibitors and has demonstrated its therapeutic capacity in an animal model of lung disease (29). Caution is, however, warranted in the long-term use of ECE inhibitors, considering their deleterious characteristics of increasing the β amyloide peptide in a mouse model of Alzheimer's disease (30). Another emerging enzymatic target in the ET-1 pathway is chymase. NK3201, a chymase inhibitor, was experimentally shown to suppress bleomycin-induced pulmonary fibrosis in the hamster (31). Thus far, however, the most successful therapy has been the employment of nonselective ET-1 receptor antagonists. Bosentan, a widely established dual endothelin receptor antagonist, is currently the treatment option that holds the most promise for patients with IPF. Novel ET antagonists with more lipophylic profiles such as Macitentan, a dual endothelin antagonist that helps to suppress a variety of endothelin-induced cardiovascular diseases (32), are also emerging.

Bosentan is a potent dual ET_A and ET_B receptor antagonist that has already demonstrated its safety and efficacy in increasing the survival of patients with pulmonary hypertension (1). Its safety and tolerability specifically in patients with IPF has also been recently assessed, with favorable results (33). Since the profibrotic effects of ET-1 are mediated by both endothelin receptor subtypes, administration of a nonselective ET receptor antagonist is an effective approach toward decreasing molecular profibrotic activities such as collagen I and III deposition (1) and preventing pulmonary scarring (33). Indeed, when bosentan was given to the aforementioned rats with bleomycin-induced pulmonary fibrosis, similar effects were observed (6).

The bosentan use in interstitial lung disease (BUILD) clinical trials are a source of optimism for finally attaining an effective treatment for IPF. BUILD-1 was a phase I international, prospective, double-blind, randomized, placebo-controlled parallel group study (34). This trial found that when compared with 84 patients on placebo, the 74 patients given bosentan demonstrated a trend of delayed time to death and progression of disease, particularly in the subgroup of biopsy-proven disease (34). The BUILD-2 study, also a double-blind, randomized, placebo-controlled study, assessed bosentan in interstitial lung disease secondary to systemic sclerosis in 163 patients (22). The results of this study point to no significant effects of bosentan in these patients (22). However, certain methods used in the BUILD-2 study may have impacted the results, such as inappropriate markers for treatment improvement, a population with inappropriate disease progression, and short duration of the study (35). BUILD-3, a phase III clinical trial, is currently in progress (35). The results of this study in the coming years may significantly influence the manner in which IPF is treated.

CONCLUSIONS

ET-1, through its molecular and cellular interactions, is clearly implicated in the fibrosis of the lung. In diseased states, resident and inflammatory cells increase the local levels of ET-1, perhaps contributing to further ET-1 release via autocrine and paracrine loops. Augmented production of ET-1 in different pulmonary cells may contribute to the excessive fibrosis seen in IPF. The future of treating fibrotic disease appears to lie in further understanding what differentiates normal from pathologic wound repair, discovering the multiple roles of profibrotic compounds such as ET-1, and in properly managing their far-reaching effects.

Conflict of Interest Statement: None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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